thereby obviating the rejection of claim 13 under 35 USC 112, second paragraph.

The Examiner rejected claims 1, 3, 5, 7 and 10 to 17 under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In this regard, the Examiner referred to the term "substantially" in claim 1.

The term "substantially free" is often used in chemical cases to denote the absence of a material with the possibility of contamination by very minor amounts of material. It is in this established sense that the terminology is used. However, in the interests of expediting prosecution, the term "substantially" has been removed from claim 1.

Having regard to this amendment, it is submitted that claims 1, 3, 5, 7 and 10 to 17 are no longer open to rejection under 35 USC 112, second paragraph.

The specific withdrawal of rejection of claims 1, 3, 5, 7, 12 to 14 and 17 under 35 USC 103 over Fredericksen et al in view of Jackson is acknowledged.

The Examiner maintains rejection of claims 10 and 11 under 35 USC 103 as being unpatentable over Fredericksen et al in view of Jackson et al and further in view of Gotto and extended the rejection to claims 1, 3, 5, 7, 12 to 14 and 17. Reconsideration is requested for the following reasons.

The present invention is directed to a specific process for the preparation of an agglutinogen preparation which comprises fimbrial agglutinogens 2 and 3 free from agglutinogen 1. This process requires that the specific combination of steps recited in claim 1. The Fredericksen et al reference relates to the preparation of agglutinogen 3 from *B. pertussis*. This reference is entirely silent with respect to the provision of a preparation containing agglutinogen 2 and 3 free from agglutinogen 1. The reference describes only the preparation of agglutinogen 3. In this respect, in the Final Action, the Examiner states:

> "Contrary to the applicants assertions the procedure of Fredericksen et al would have resulted in a combination of agglutinogens 2 and 3 because the procedure is essentially identical to applicants procedure". (Emphasis added).

As will clearly be demonstrated below, while there may be elements of similarity between applicants specifically-defined procedure and the Fredericksen et al

procedure, they are certainly not "essentially identical" as asserted by the Examiner. In any event, the fact remains that Fredericksen et al is entirely silent as to the potential presence of agglutinogen 2. Since this material was a known material at the time of the Frederickson et al reference, a person skilled in the art would expect Fredericksen et al to describe the presence of such material, if indeed it were present.

The Examiner applies hindsight to the analysis of the prior art in relation to the claimed process. The processes are different (the rejection is under 35 USC 103 and not 35 USC 102) and hence a presumption cannot be made as to an essential component of the product of applicants process. The processes are not "essentially identical" but, in fact, quite different. There is no evidence to suggest that the Fredericksen et al process produces a material containing agglutinogen 2 but rather the evidence is (the silence of Fredericksen et al in this respect) that the procedure of Fredericksen et al produces only agglutinogen 3, the stated product of his process.

The initial step of applicants process is the provision of a cell paste of the *Bordetella* strain from which the agglutinogen preparation is to be formed, such as *Bordetella pertussis* (claim 17). The fimbrial agglutinogens 2 and 3 then are selectively extracted from the cell paste by dispersing the same in a buffer comprising about 1 to about 5 molar urea which produces a first supernatant containing the fimbrial agglutinogens 2 and 3 and a first residual precipitate. The supernatant then is separated from the residual precipitate.

The supernatant then is incubated to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3 and a second precipitate containing non-fimbrial agglutinogen contaminants which may have been extracted during the extraction step and comprising mainly agglutinogen 1.

The clarified supernatant then is concentrated to produce a crude fimbrial agglutinogen solution. Such concentration is effected by first precipitating the fimbrial agglutinogens 2 and 3 from a clarified supernatant by the addition of polyethylene glycol, separation of the precipitated fimbrial agglutinogens 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3. This crude fimbrial agglutinogen solution then is purified, such as by column chromatography (claim 12), to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3 substantially

free from agglutinogen 1.

It is submitted that this process is quite different from the prior art and is not rendered obvious by the combination of cited prior art. It has already been pointed out above that Fredericksen et al is solely concerned with the preparation of agglutinogen 3 from *B. pertussis*. Neither subsidiary reference alters the clear teaching of Fredericksen et al in this regard.

In Fredericksen et al, bacterial *B. pertussis* cells are resuspended in PBS containing sodium chloride and then the suspension is added to ten volumes of acetone to effect dehydration of the bacteria, followed by evaporation of the acetone to obtain a dry powder. Applicants process employs no such steps. Following the dehydration step, in Fredericksen et al, the powder is resuspended in PBS and whole cells and larger fragments are removed from the suspension by centrifugation. The resuspension and centrifugation step are effected twice more to result in a crude extract.

Applicants procedure differs significantly from this procedure in that applicants effect a selective extraction of fimbrial agglutinogens 2 and 3 from a cell paste by dispersing the cell paste in a buffer comprising about 1 to about 6 molar urea which produces an aqueous supernatant which contain the agglutinogen 2 and 3 and a residual precipitate. There is no organic solvent, such as the acetone employed by Fredericksen et al, to effect dehydration of the bacteria. The applicants employ an aqueous extraction step to selectively solubilize the agglutinogen 2 and 3. In addition, applicants procedure does not require resuspension of a powered extract nor the removal of whole cells and larger fragments from such suspension, nor repetition of such steps. Rather applicant effects an initial selective extraction step for agglutinogens 2 and 3 by the use of an aqueous buffer, which successfully separates the agglutinogens 2 and 3 from the cellular fragments.

Applicants do not employ the elaborate procedure of Fredericksen et al, involving dehydration of bacteria, evaporation of organic solvent, separation, resuspension and centrifugation. As already mentioned, Fredericksen et al is silent as to an agglutinogen 2 component. In the Final Action, the Examiner states:

> "With respect to the use of urea, it remains the position of the Examiner that the prior art procedure employing acetone extraction is <u>functionally equivalent</u>, and

therefore within the level of ordinary skill in the art." (Emphasis added)

One process, Fredericksen et al uses an organic solvent, evaporation to dryness and three-fold resuspension and centrifugation while the other, claim 1, uses a simple aqueous urea extraction step followed by separation of supernatant from residual material. Once again, it is emphasized that the Fredericksen et al process is intended to produce agglutinogen 3 alone whereas applicants process is designed to produce a mixture of agglutinogens 2 and 3.

It is not clear what the Examiner means by the term "functionally equivalent". If the Examiner is applying the "way, means, result" test of the doctrine of equivalents, the process steps are clearly quite different. In addition, the Examiner chooses to ignore the multiple resuspension and centrifugation steps that are essentially required by Fredericksen et al in addition to the steps of dehydration of bacteria using acetone and evaporation of acetone to provide a powder.

Following the above-described steps, applicants then incubate the aqueous suspension at a temperature of about 75° to about 85°C for about 10 to about 60 minutes to produce a clarified supernatant containing the fimbrial agglutinogens 2 and 3 and a second precipitate containing non-fimbrial agglutinogen contaminants. It is noted that Fredericksen et al treat the crude extract from the multiple resuspensions and centrifugations of the powdered extract by heating at 80°C for 5 minutes followed by removal of aggregated material. It is noted that applicants heat treatment process to remove non-fimbrial agglutinogen contaminants (agglutinogen 1) and produce a clarified supernatant is effected for a longer period of time than the heat treatment step in Fredericksen et al and, of course, Fredericksen et al is silent as to the effect of this heat treatment step, other than to identify the formation of a precipitate. This reference does not in any manner indicate that the incubation step results in the removal of non-fimbrial agglutinogen contaminants to leave fimbrial agglutinogens 2 and 3 in a clarified supernatant, as in applicant's process.

In the Final Action, the Examiner states:

"The heat treatment procedure of Fredericksen et al is <u>equivalent</u> to that of applicants in that the methods are <u>substantively</u> the same in view of the similar temperatures employed, the limitation of "about"

> occurs in applicant's claims, and purpose for which this step is employed are identical". (Emphasis added)

The Examiners reference to the term "about" appearing in applicants claims is misleading. The term "about" is conventionally used in chemical patent applications, such as this one, to define numerical ranges and that practice has been cited with approval in many court decisions. The term "about" is intended to permit some leeway in the recited numerical range. However, the clearly recited 5 minutes heating time in Fredericksen et al is clearly not the minimum "about 10 minutes" used by applicants. How can heating for about 10 to about 60 minutes at about 75° to about 85°C be considered to "equivalent" and "substantially the same" as heating at 80°C for 5 minutes, as recited by Fredericksen et al? The Examiner offers no justification in this regard.

Under the heading of "Heat Treatment of Crude Extract", it is stated by Fredericksen et al:

> "The crude extract was heated at 80°C for 5 min and aggregated material removed by centrifugation at 20,000 xg for 30 min."

Having regard to this statement, the source of the Examiners statement that the "purpose for which this step is employed are identical" is obscure. If the Examiner has specific knowledge in this respect, he is required to provide this information by way of an Affidavit under 37 CFR 1.107(b). Otherwise, it is not clear how the Examiner can assert that the Fredericksen et al heating step effected on his crude extract is identical in purpose to applicants heat treatment of supernatant to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3 and a second preparation containing non-fimbrial agglutinogen contaminants. The Fredericksen et al reference is entirely silent as to the agglutinogens 2 and 3 and the nature of the aggregated material.

In applicants process, the fimbrial agglutinogens 2 and 3 are precipitated from the clarified supernatant by the addition of a polyethylene glycol to the clarified supernatant, followed by separation of the precipitated fimbrial agglutinogens and subsequent solubilization of the precipitated fimbrial agglutinogens to form a concentrate which is a crude agglutinogen solution containing agglutinogens 2 and 3. The agglutinogens 2 and 3 then are purified from the crude solution to produce the fimbrial agglutinogen preparation

comprising agglutinogens 2 and 3 from which agglutinogen 1 is absent.

Fredericksen et al again take a different path. In Fredericksen et al, the supernatant which results from the heat treatment is subject to an ammonia sulphate precipitation step by adding saturated ammonia sulphate solution at 40°C to the supernatant to 25% saturation. After 60 minutes "or more", the preparation is collected by centrifugation and dissolved in PBS followed by further centrifugation. The fraction which results is subjected to gel filtration chromatography for further purification. Fredericksen et al, therefore, at this stage of their process, use an ammonia sulphate precipitation whereas applicants use polyethylene glycol to effect precipitation of agglutinogens 2 and 3.

The Examiner states in the Final Action:

> "Other steps of the process procedure parallel those by applicants for the reasons cited in the last Office Action" (Emphasis Added)

In the only portion of the prior Office Action which appears to address the specific step of PEG precipitation, the Examiner states:

> "Gotto shows that PEG and ammonium sulphate are equivalent reagents for protein precipitation and may be used in protein purification schemes for *B. pertussis*. Therefore, because these two protein precipitation reagents were art-recognised equivalents at the time the invention was made, one of ordinary skill in the art would have found it obvious to substitute PEG for ammonium sulphate".

As was pointed out to the Examiner in response to the first Office Action, Gotto is concerned with a process for the preparation of outer membrane protein of molecular weight about 69,000 daltons, namely pertactin, which is the same end product as the Jackson et al procedure. The Gotto reference discloses a multistep operation for producing such a product by extracting cells and utilizing a dye ligand chromatographic support for separation of the 69,000 dalton outer membrane protein. In the specific description of the procedure involved, it is indicated in column 7, lines 57 to 63 that the protein extract resulting from incubation of the cells in an extract medium is contacted with polyethylene glycol to effect precipitation of the protein extract so as to provide a precipitate fraction which is rich in the 69K protein, i.e. the product of interest. It is further indicated that other reagents commonly used to precipitate proteins, including ammonium sulphate or organic

solvent, such as ethanol or acetone, may be employed. All that this passage indicates is that there are a number of reagents which have been used or suggested to be used to effect precipitation of proteins from aqueous media and polyethylene glycol happens to be the reagent of choice for the precipitation of the 69K protein from the extract material that is produced in the Gotto reference. This reference is wholly silent as to any preparative procedure involving agglutinogens. Simply because the 69K protein of Gotto et al and agglutinogens 2 and 3 are proteins recoverable from *B. pertussis* does not mean that this procedure or steps in such procedure are "equivalent" or even "parallel".

The disclosure of Gotto in no way suggests that polyethylene glycol may be a suitable reagent for the precipitation of agglutinogens, since the Gotto reference is entirely silent as to agglutinogens and certainly is in no way directed to a process for the preparation of agglutinogens 2 and 3 separate from agglutinogen 1 but rather, as in the case of the Jackson et al reference, is concerned with a process for producing a 69K outer membrane protein (pertactin) of *B. pertussis*. No other product from the processing of the *B. pertussis* is desired either in the Jackson et al or Gotto process. Any other material which may be present in the extract is undesirable and is removed and discarded in a procedure to obtain a purified form of the pertactin 69K protein.

The applicants have analysed the relationship of the process of claim 1 to Fredericksen et al and the other prior art on a step-by-step basis, since this is the analysis made by the Examiner. The Examiner has brought to bear that hindsight of the present invention to this analysis and has determined individual steps of applicants process to be "equivalent" and "parallel" to certain steps in Fredericksen et al. The analysis should be a consideration of the invention as defined by applicants claims as a whole in relation to the totality of the teachings of the prior art.

The Examiner is wholly silent to the Final Action as to any potential relevance of the Fredericksen et al and Gotto et al references, other than by reference to "the last Office Action" without other citation to assist the applicants in considering and rebutting the Examiners position. This is all the more so since the Examiner states that he has simply extended a rejection based on these references from certain subclaims to all other claims, except claims 15 and 16, without further explanation.

Applicants have described above how the process of claim 1 differs

both in terms of product obtained and specific process steps adopted from the process described in Fredericksen et al. The applicants have discussed above the irrelevance of Gotto et al. Jackson et al is similarly irrelevant. The Examiner has offered no rebuttal. In the first Office Action, the Examiner indicates that relevance on Jackson et al is as follows:

> "Jackson et al teaches the purification of pertactin from *B. pertussis* by placing the cells in a 4m solution of urea, then separating the precipitate from the supernatant by centrifugation. In order to obtain pertactin from the supernatant it is subjected to ultrafiltration. Most importantly, Jackson et al. teaches that the supernatant contains agglutinogens which are separated from the pertactin by the ultrafiltration. See column 4, lines 12-20."

As pointed out in response to the first Office Action, The Examiner refers to the Jackson reference for a teaching of purification of pertactin from pertussis in which cells are extracted with urea followed by separation of the precipitate from the supernatant by centrifugation. As the Examiner correctly points out, the whole thrust of the Jackson et al reference is directed to the preparation of purified pertactin and, in one embodiment of the procedures described, pertactin is extracted from the grown cells, using urea followed by removal of cell debris by centrifugation. Ultrafiltration of the supernatant is described as being effected to remove high molecular weight proteins with agglutinogens being indicated to be among the high molecular weight proteins which are removed by such procedure.

It is clear that the Jackson et al reference in no way suggests any further processing or procedure for separation of the agglutinogens and certainly does not disclose or suggest a procedure which is able to produce an agglutinogen preparation comprising agglutinogens 2 and 3 substantially free from agglutinogen 1. All that the Jackson et al reference is concerned with is preparing a solution from which pertactin may be separated in a pure form.

Jackson et al effect a cell extraction procedure using four molar urea and ultrafiltration to remove high molecular weight contaminants from that solution. It is submitted that such a procedure falls far short of any suggestion of a preparative procedure for obtaining agglutinogens 2 and 3 free from agglutinogen 1. At best, Jackson et al suggest that one procedure whereby pertactin may be isolated involves an initial cell extraction using aqueous urea, followed by a separation step which

discards any agglutinogens and other high molecular weight proteins which may be present in the extract.

The Examiner offers no rebuttal.

It is clear, therefore, that applicants process claim 1, when considered as a whole, is clearly patentably distinguished from the combination of Fredericksen et al, Jackson et al and Gotto et al. Claims 3, 5, 7, 10 to 14 and 17 are dependent, directly or indirectly, on claim 1, and for this reason alone are patentable over the applied art. These claims refer to subsidiary features of the process. For example, claims 10 and 11 recite specific conditions for effecting the PEG precipitation. Not only is the PEG precipitation step in a procedure for preparation of agglutinogens not present in the art, but also such specific conditions alone are not disclosed.

Having regard to the above, it is submitted that claims 1, 3, 5, 7, 10 to 14 and 17 are clearly patentable over the applied art and the rejection thereof under 35 USC 103 as being unpatentable over Fredericksen et al in view of Jackson et al and Gotto et al, should be withdrawn.

The Examiner maintained rejection of claims 15 and 16 under 35 USC 103 over Fredericksen et al in view of Jackson et al and further in view of Kieff et al. Reconsideration is requested for the following reasons.

Claims 15 and 16 are dependent indirectly on claim 1. The applicants had urged, in response to the prior Office Action, that, having demonstrated the patentability of claim 1, then these claims too were patentable, having regard to the manner in which the Examiner relied on Kieff et al. In this regard, the Examiner relies on Kieff et al solely for a teaching of a vaccine composition of a protein absorbed on alum. Applicants admit that it is known in the art to absorb vaccine components on alum for the purpose of providing immunogenic compositions. Claims 15 and 16 are directed specifically to absorption of the fimbrial agglutinogen preparation comprising the agglutinogens 2 and 3 free from agglutinogen 1 on a mineral salt adjuvant, such as alum. The applicants claim no separate inventive step with respect to such recitation.

In the Final Action, the Examiner states:

> "Applicants assert that this rejection should fall in view of their demonstration of the patentability of claim 1 over the prior art. However, the rejection of claim 1 over the prior art has been maintained, and therefore claims 15 and 16 remain rejected."

However, the rejection of claim 1 has changed from the first Office Action. In the Final Action, the rejection of claim 1 is under 35 USC 103 as being unpatentable over the combination of Fredericksen et al with Jackson et al and Gotto et al. The Examiners rejection of claims 15 and 16 is on the combination of Fredericksen et al , Jackson et al and Keiff et al, i.e, without Gotto et al. Accordingly, it is unclear which rejection the Examiner is making.

In any event, suffice it to say that applicants have demonstrated above that claim 1 is patentable over the combination of prior art applied against that claim. As discussed above, Kieff et al is not relied on to remedy the defects of such combination , but rather for disclosure of the specific features of claims 15 and 16.

Accordingly, it is submitted that claims 15 and 16 are patentable over the applied art and hence the rejection of claims 15 and 16 under 35 USC 103 as being unpatentable over Fredericksen et al in view of Jackson et al and Kieff et al, should be withdrawn.

Entry of this Amendment after Final Action is requested, in that the application thereby is placed in condition for allowance. In the event that the Examiner considers one or more ground of rejection to remain, the Amendment nevertheless should be entered, since the issues for appeal are reduced.

In the event that the Examiner believes that further revisions are required to the claim language to place the claims in an allowable form, the Examiner is urged to contact the applicants representative, Mr. Michael I. Stewart, collect, at the number given below, with a view to arriving at mutually-acceptable language.

It is believed that this application now is in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

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